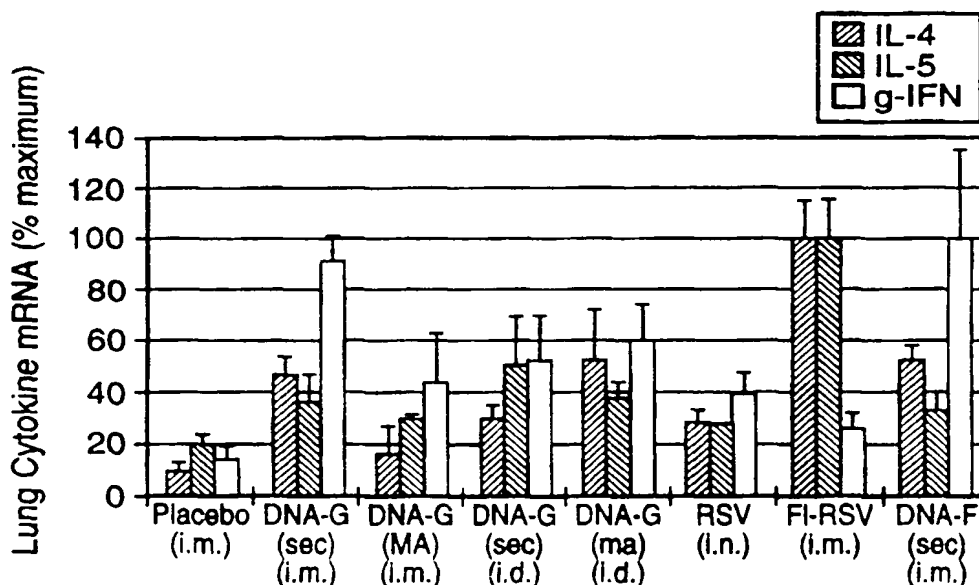


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(54) Title: NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS



(57) Abstract

Non-replicating vectors, such as plasmid vectors, containing a nucleotide sequence coding for a G protein of respiratory syncytial virus (RSV) and a promoter for such sequence, preferably a cytomegalovirus promoter, are described. Such vectors also may contain a further nucleotide sequence located adjacent to the RSV G protein encoding sequence to enhance the immunoprotective ability of the RSV G protein when expressed *in vivo*. Such non-replicating vectors may be used to immunize a host, including a human host, against RSV infection by administration thereto. Such non-replicating vectors also may be used to produce antibodies for detection of RSV infection in a sample.

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TITLE OF INVENTIONNUCLEIC ACID VACCINES ENCODING G PROTEIN OF  
RESPIRATORY SYNCYTIAL VIRUS

5

FIELD OF INVENTION

The present invention is related to the field of respiratory syncytial virus (RSV) vaccines and is particularly concerned with vaccines comprising nucleic acid sequences encoding the attachment (G) protein of RSV.

BACKGROUND OF INVENTION

Respiratory syncytial virus (RSV), a negative-strand RNA virus belonging to the *Paramyxoviridae* family of viruses, is the major viral pathogen responsible for bronchiolitis and pneumonia in infants and young children (ref. 1 - Throughout this application, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure. Acute respiratory tract infections caused by RSV result in approximately 90,000 hospitalizations and 4,500 deaths per year in the United States (ref. 2). Medical care costs due to RSV infection are greater than \$340 M annually in the United States alone (ref. 3). There is currently no licensed vaccine against RSV. The main approaches for developing an RSV vaccine have included inactivated virus, live-attenuated viruses and subunit vaccines.

A protective immune response against RSV is thought to require the induction of neutralizing antibodies against the surface fusion (F) and attachment (G) glycoproteins (ref. 4). In addition, cytotoxic T lymphocytes (CTL) responses are involved in viral clearance. The F protein is conserved amongst the RSV A

and B subgroups.

The G protein (33 kDa) of RSV is heavily O-glycosylated giving rise to a glycoprotein of apparent molecular weight of 90 kDa (ref. 5). Two broad subtypes  
5 of RS virus have been defined: A and B (ref. 6). The major antigenic differences between these subtypes are found in the G glycoprotein (refs. 3, 7).

The use of RSV proteins as vaccines may have obstacles. Parenterally administered vaccine candidates  
10 have so far proven to be poorly immunogenic with regard to the induction of neutralizing antibodies in seronegative chimpanzees. The serum antibody response induced by these antigens may be further diminished in the presence of passively acquired antibodies, such as  
15 the transplacentally acquired maternal antibodies which most young infants possess. A subunit vaccine candidate for RSV consisting of purified fusion (F) glycoprotein from RSV infected cell cultures and purified by immunoaffinity or ion-exchange chromatography has been  
20 described (ref. 8). Parenteral immunization of seronegative or seropositive chimpanzees with this preparation was performed and three doses of 50 µg were required in seronegative animals to induce an RSV serum neutralizing titre of approximately 1:50. Upon  
25 subsequent challenge of these animals with wild-type RSV, no effect of immunization on virus shedding or clinical disease could be detected in the upper respiratory tract. The effect of immunization with this vaccine on virus shedding in the lower respiratory tract  
30 was not investigated, although this is the site where the serum antibody induced by parenteral immunization may be expected to have its greatest effect. Safety and immunogenicity studies have been performed in a small number of seropositive individuals. The vaccine was  
35 found to be safe in seropositive children and in three

seronegative children (all > 2.4 years of age). The effects of immunization on lower respiratory tract disease could not be determined because of the small number of children immunized. One immunizing dose in seropositive children induced a 4-fold increase in virus neutralizing antibody titres in 40 to 60% of the vaccinees. Thus, insufficient information is available from these small studies to evaluate the efficacy of this vaccine against RSV-induced disease. A further problem facing subunit RSV vaccines is the possibility that inoculation of seronegative subjects with immunogenic preparations might result in disease enhancement. In the 1960's, vaccination of infants with a formalin-inactivated RSV preparation (FI-RSV) resulted in enhanced lung disease upon subsequent exposure to live virus, also referred to as immunopotentialization (refs. 9, 10). These vaccinees developed strong serological responses, but were not protected against infection and some developed severe, occasionally fatal respiratory tract disease upon natural infection. Although precise mechanisms remain unknown, it has been suggested that this form of immune enhancement might reflect either structural alterations of RSV antigens (ref. 11), residual serum and/or cellular contaminants (ref. 12), a specific property of the viral attachment (G) protein (refs. 13,14) or an imbalanced cell-mediated immune response (refs. 13,15). It has been demonstrated that the FI-RSV vaccine induced a TH2-type immune response in mice whereas immunization with live RSV, which does not cause immunopotentialization, elicits a TH1 response (ref.15).

In some studies, the immune response to immunization with a synthetic RSV FG fusion protein resulted in disease enhancement in rodents resembling that induced by a formalin-inactivated RSV vaccine.

Immunization of mice with a recombinant vaccinia virus expressing the RSV G protein resulted in G-specific T cell responses in the lungs which are exclusively recruited from the CD4+T cell sublineage and are  
5 strongly Th2-biased. G-specific T cells induce lung haemorrhage, pulmonary neutrophil recruitment (shock lung), intense pulmonary eosinophilia, and sometimes death in the adoptively transferred murine recipients (ref. 14). The association of immunization with disease  
10 enhancement using certain vaccine preparations including non-replicating antigens suggests caution in their use as vaccines in seronegative humans.

Live attenuated vaccines against disease caused by RSV may be promising for two main reasons. Firstly,  
15 infection by a live vaccine virus induces a balanced immune response comprising mucosal and serum antibodies and cytotoxic T-lymphocytes. Secondly, infection of infants with live attenuated vaccine candidates or naturally acquired wild-type virus is not associated  
20 with enhanced disease upon subsequent natural reinfection. It will be challenging to produce live attenuated vaccines that are immunogenic for younger infants who possess maternal virus-neutralizing antibodies and yet are attenuated for seronegative  
25 infants greater than or equal to 6 months of age. Attenuated live virus vaccines also have the risks of residual virulence and genetic instability.

Injection of plasmid DNA containing sequences encoding a foreign protein has been shown to result in  
30 expression of the foreign protein and the induction of antibody and cytotoxic T-lymphocyte (CTL) responses to the antigen in a number of studies (see, for example, refs. 16, 17, 18). The use of plasmid DNA inoculation to express viral proteins for the purpose of  
35 immunization may offer several advantages over the

strategies summarized above. Firstly, DNA encoding a viral antigen can be introduced in the presence of antibody to the virus itself, without loss of potency due to neutralization of virus by the antibodies.

5 Secondly, the antigen expressed in vivo should exhibit a native conformation and the appropriate glycosylation. Therefore, the antigen should induce an antibody response similar to that induced by the antigen present in the wild-type virus infection. In contrast, some

10 processes used in purification of proteins can induce conformational changes which may result in the loss of immunogenicity of protective epitopes and possibly immunopotential. Thirdly, the expression of proteins from injected plasmid DNAs can be detected in vivo for a

15 considerably longer period of time than that in virus-infected cells, and this has the theoretical advantage of prolonged cytotoxic T-cell induction and enhanced antibody responses. Fourthly, in vivo expression of antigen may provide protection without the need for an

20 extrinsic adjuvant.

The ability to immunize against disease caused by RSV by administration of a DNA molecule encoding an RSV G protein was unknown before the present invention. In particular, the efficacy of immunization against RSV

25 induced disease using a gene encoding a secreted form of the RSV G protein was unknown. Infection with RSV leads to serious disease. It would be useful and desirable to provide isolated genes encoding RSV G protein and non-replicating vectors, including plasmid vectors, for in

30 vivo administration and for use in immunogenic preparations, including vaccines, for protection against disease caused by RSV and for the generation of diagnostic reagents and kits. In particular, it would be desirable to provide vaccines that are immunogenic

35 and protective in humans, including seronegative

infants, that do not cause disease enhancement (immunopotentialiation).

#### SUMMARY OF INVENTION

The present invention relates to a method of  
5 immunizing a host against disease caused by respiratory syncytial virus, to non-replicating vectors containing nucleic acid molecules used in immunogenic compositions for such purpose, and to diagnostic procedures utilizing the vectors and nucleic acid molecules. In particular,  
10 the present invention is directed towards the provision of nucleic acid vaccines encoding the G protein of respiratory syncytial virus.

In accordance with one aspect of the invention, there is provided an immunogenic composition for in vivo  
15 administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G  
20 protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

25 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host, and

a pharmaceutically-acceptable carrier therefor.

30 The first nucleotide sequence may be that which encodes a full-length RSV G protein. The first nucleotide sequence may comprise the nucleotide sequence shown in Figure 2 (SEQ. ID No: 1) or encode a full length RSV G protein having the amino acid sequence  
35 shown in Figure 2 (SEQ. ID no: 2).



Alternatively, the first nucleotide sequence may be that which encodes an RSV G protein from which the transmembrane coding sequence and sequences upstream thereof are absent. The first nucleotide sequence  
5 encoding the truncated RSV G protein may comprise the nucleotide sequence shown in Figure 3 (SEQ. ID no: 3) or may comprise a nucleotide sequence encoding the truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID no: 4). The lack of  
10 expression of the transmembrane region results in a secreted form of the RSV G protein.

The non-replicating vector may further comprise a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of the first  
15 nucleotide sequence. The signal peptide encoding sequence may encode the signal peptide of human tissue plasminogen activator.

The promoter sequence may be an immediate early cytomegalovirus (CMV) promoter. The second nucleotide  
20 sequence may comprise the human cytomegalovirus Intron A.

The non-replicating vector generally is a plasmid vector. Plasmid vectors encoding the G protein and included in the immunogenic composition provided by this  
25 aspect of the invention may specifically be pXL5 or pXL6, constructed and having their characterizing elements, as seen in Figures 4 or 5, respectively.

In accordance with a further aspect of the present invention, there is provided a method of immunizing a  
30 host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to the host an effective amount of a non-replicating vector comprising:

a first nucleotide sequence encoding an RSV G  
35 protein or a RSV G protein fragment that generates

antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

- 5 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host.

The immunization method may be effected to induce a  
10 balanced Th1/Th2 immune response.

The present invention also includes a novel method of using a gene encoding respiratory syncytial virus (RSV) G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G  
15 protein, to protect a host against disease caused by infection with respiratory syncytial virus, which comprises:

isolating the gene;

operatively linking the gene to at least one  
20 control sequence to produce a non-replicating vector, said control sequence directing expression of the RSV G protein when said vector is introduced into a host to produce an immune response to the RSV G protein, and

introducing the vector into the host.

- 25 The procedure provided in accordance with this aspect of the invention may further include the step of:

operatively linking the gene to an immunoprotection enhancing sequence to produce an enhanced immunoprotection by the RSV G protein in the host,  
30 preferably by introducing the immunoprotection enhancing sequence between the control sequence and the gene, including introducing immunostimulatory CpG sequences in the vector.

In addition, the present invention includes a  
35 method of producing a vaccine for protection of a host

against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isolating a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates  
5 antibodies that specifically react with RSV G protein,

operatively linking the first nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of the RSV G protein when introduced into a  
10 host to produce an immune response to the RSV G protein when expressed in vivo from the vector in a host,

operatively linking the first nucleotide sequence to a second nucleotide sequence to increase expression of the RSV G protein in vivo from the vector in a host,  
15 and

formulating the vector as a vaccine for in vivo administration.

The vector may be a plasmid vector selected from pXL5 and pXL6. The invention further includes a vaccine  
20 for administration to a host, including a human host, produced by this method.

As noted previously, the vectors provided herein are useful in diagnostic applications. In a further aspect of the invention, therefore, there is provided a  
25 method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising the steps of:

(a) immunizing a host with a non-replicating vector to produce antibodies specific for the RSV G protein, the non-replicating vector comprising a  
30 first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to  
35 the first nucleotide sequence for expression of the

RSV G protein in the host and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein in vivo from the vector in the host;

5

(b) isolating the RSV G protein-specific antibodies;

(c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in the sample and the RSV G protein-specific antibodies; and

10

(d) determining production of the complexes.

The non-replicating vector employed to elicit the antibodies may be a plasmid vector pXL5 or pXL6.

15

The invention also includes a diagnostic kit for detecting the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising:

(a) a non-replicating vector capable of generating antibodies specific for the RSV G protein when administered to a host, said non-replicating vector comprises a first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in a host, and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein in vivo from the vector in the host;

20

25

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(b) isolation means to isolate the RSV G protein specific antibodies;

(c) contacting means to contact the isolated RSV G protein-specific antibodies with the sample to produce a complex comprising any RSV G protein

35

present in the sample and RSV G protein specific antibodies; and

(d) identifying means to determine production of the complex.

5 The present invention further is directed to a method for producing antibodies specific for a G protein of a respiratory syncytial virus (RSV) comprising:

10 (a) immunizing a host with an effective amount of a non-replicating vector to produce RSV G-specific antibodies, said non-replicating vector comprising:

15 a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

20 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host; and

(b) isolating the RSV G specific antibodies from the host.

25 The present invention is also directed to a method for producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV), comprising the steps of:

30 (a) constructing a vector comprising a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in the host and a second nucleotide

35

sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein when in vivo from the vector in a host;

- 5 (b) administering the vector to at least one mouse to produce at least one immunized mouse;
- (c) removing B-lymphocytes from the at least one immunized mouse;
- (d) fusing the B-lymphocytes from the at least
- 10 one immunized mouse with myeloma cells, thereby producing hybridomas;
- (e) cloning the hybridomas;
- (f) selecting clones which produce anti-RSV G protein antibody;
- 15 (g) culturing the anti-RSV G protein antibody-producing clones; and
- (h) isolating anti-RSV G protein monoclonal antibodies.

Such monoclonal antibodies may be used to purify RSV G  
20 protein from virus.

In this application, the term "RSV G protein" is used to define a full-length RSV G protein, such proteins having variations in their amino acid sequences including those naturally occurring in various strains  
25 of RSV, a secreted form of RSV G protein lacking a transmembrane region, as well as functional analogs of the RSV G protein. In this application, a first protein is a "functional analog" of a second protein if the first protein is immunologically related to and/or has  
30 the same function as the second protein. The functional analog may be, for example, an immunologically-active fragment of the protein or an immunologically-active substitution, addition or deletion mutant thereof.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further understood from the following General Description and Examples with reference to the Figures of the accompanying drawings, 5 in which:

Figure 1 illustrates a restriction map of the gene encoding a G protein of respiratory syncytial virus (RSV);

10 Figure 2 illustrates the nucleotide sequence of a gene encoding a membrane bound form of the G protein of respiratory syncytial virus (SEQ ID No: 1) as well as the amino acid sequence of the RSV G protein encoded thereby (SEQ ID No: 2);

15 Figure 3 illustrates the nucleotide sequence of a gene encoding the secreted form of the RSV G protein lacking the transmembrane domain (SEQ ID No: 3) as well as the amino acid sequence of a truncated RSV G protein lacking the transmembrane domain encoded thereby (SEQ ID No: 4);

20 Figure 4 shows the construction of plasmid pXL5 containing a gene encoding a full-length membrane attached form of the RSV G protein and containing the CMV Intron A sequence;

25 Figure 5 shows the construction of plasmid pXL6 containing a gene encoding a secreted form of the RSV G protein lacking the transmembrane domain and containing the CMV Intron A sequence as well as a nucleotide sequence encoding a signal peptide of the human tissue plasminogen activator (TPA);

30 Figure 6 shows the nucleotide sequence for the plasmid VR-1012 (SEQ ID No. 5);

Figure 7 shows the nucleotide sequence for the 5' untranslated region and the signal peptide of the human tissue plasminogen activator (TPA) (SEQ. ID no: 6) and

35 Figure 8 shows the lung cytokine expression profile

in DNA immunized mice after RSV challenge.

GENERAL DESCRIPTION OF INVENTION

As described above, the present invention relates generally to polynucleotide, including DNA, immunization  
5 to obtain protection against infection by respiratory syncytial virus (RSV) and to diagnostic procedures using particular non-replicating vectors. In the present invention, several recombinant plasmid vectors were constructed to contain a nucleotide sequence encoding an  
10 RSV G protein.

The nucleotide sequence of the full length RSV G gene is shown in Figure 2 (SEQ ID No: 1). Certain constructs provided herein include the nucleotide sequence encoding the full-length RSV G (SEQ ID No: 2)  
15 protein while others include an RSV G gene modified by deletion of the transmembrane coding sequence and nucleotides upstream thereof (see Figure 3, SEQ ID No: 3), to produce a secreted or truncated RSV G protein lacking the transmembrane domain (SEQ ID No. 4).

20 The nucleotide sequence encoding the RSV G protein is operatively coupled to a promoter sequence for expression of the encoded RSV G protein in vivo. The promoter sequence may be the human immediately early cytomegalovirus (CMV) promoter. This promoter is  
25 described in ref. 19. Any other convenient promoter may be used, including constitutive promoters, such as, the Rous Sarcoma Virus LTRs, and inducible promoters, such as the metallothionin promoter, and tissue specific promoters.

30 The non-replicating vectors provided herein, when administered to an animal in the form of an immunogenic composition with a pharmaceutically-acceptable carrier, effect in vivo RSV G protein expression, as demonstrated by an antibody response in the animal to which it is  
35 administered. Such antibodies may be used herein in the



detection of RSV protein in a sample, as described in more detail below. The administration of the non-replicating vectors, specifically plasmids pXL5 and pXL6, produced anti-G antibodies, virus neutralizing  
5 antibodies, a balanced Th1/Th2 response in the lungs post viral challenge and conferred protection in mice against live RSV infection, as seen from the Examples below.

The recombinant vector also may include a second  
10 nucleotide sequence located adjacent the RSV G protein encoding nucleotide sequence to enhance the immunoprotective ability of the RSV G protein when expressed in vivo in a host. Such enhancement may be provided by increased in vivo expression, for example,  
15 by increased mRNA stability, enhanced transcription and/or translation. This additional sequence generally is located between the promoter sequence and the RSV G protein-encoding sequence. This enhancement sequence may comprise the immediate early cytomegalovirus Intron  
20 A sequence.

The non-replicating vector provided herein may also comprise an additional nucleotide sequence encoding a further antigen from RSV, an antigen from at least one other pathogen or at least one immunomodulating agent,  
25 such as a cytokine. Such vector may contain the additional nucleotide sequence in a chimeric or a bicistronic structure. Alternatively, vectors containing the additional nucleotide sequence may be separately constructed and coadministered to a host,  
30 along with the non-replicating vectors provided herein.

The non-replicating vector may further comprise a nucleotide sequence encoding a heterologous viral or eukaryotic signal peptide, such as the human tissue plasminogen activator (TPA) signal peptide, in place of  
35 the endogenous signal peptide for the truncated RSV G

protein. Such nucleotide sequence may be located immediately upstream of the RSV G encoding sequence in the vector.

The immunogenicity of the non-replicating DNA  
5 vectors may be enhanced by inserting immunostimulatory CpG sequences in the vector.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination,  
10 diagnosis and treatment of RSV infections. A further non-limiting discussion of such uses is further presented below.

#### 1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as  
15 vaccines, may be prepared from the RSV G genes and vectors as disclosed herein. The vaccine elicits an immune response in an animal which includes the production of anti-RSV G antibodies. Immunogenic compositions, including vaccines, containing the nucleic  
20 acid may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid  
25 liposome (for example, as described in WO 9324640, ref. 20) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting  
30 in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal  
35 compartment. Published PCT application WO 94/27435

describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other  
5 transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264  
10 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the  
15 layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactide-co-glycolide). Other polymers for  
20 encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

25 Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake  
30 of vaccine across the nasal mucosae. The delivery vehicle may additionally contain an absorption enhancer.

The RSV G gene containing non-replicating vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may  
35 include, water, saline, dextrose, glycerol, ethanol, and

combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof.

5 Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic  
10 compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral  
15 (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral  
20 formulations may include normally employed incipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage  
25 formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the RSV G  
30 protein and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one  
35 skilled in the art and may be of the order of about 1 µg

to about 2 mg of the RSV G gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations.

5 The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are  
10 combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

15 Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic  
20 themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen  
25 depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have been  
30 identified that enhance the immune response to antigens.

Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively  
35 commonly referred to as alum) are routinely used as

adjuvants in human and veterinary vaccines.

A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens to produce immune stimulating complexes (ISCOMS), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as monophoryl lipid A, QS 21 and polyphosphazene.

In particular embodiments of the present invention, the non-replicating vector comprising a first nucleotide sequence encoding an G protein of RSV may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The immunogenicity of the non-replicating vector may be enhanced by coadministering plasmid DNA vectors expressing cytokines or chemokines or by coexpressing such molecules in a bis-cistronic or fusion construct.

The non-replicating vector may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 21) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 22) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

## 2. Immunoassays

The RSV G genes and vectors of the present invention are useful as immunogens for the generation of anti-G antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or

procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the RSV G protein. These RSV G-specific antibodies are immobilized onto a  
5 selected surface, for example, a surface capable of binding the antibodies, such as the wells of a polystyrene microtiter plate. After washing to remove unadsorbed antibodies, a non-specific protein, such as a solution of bovine serum albumin (BSA) that is known to  
10 be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of non-specific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the  
15 surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may  
20 include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C.  
25 Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test  
30 sample and the bound RSV G specific antibodies, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

#### BIOLOGICAL MATERIALS

Certain plasmids that contain the gene encoding the  
35 RSV G protein and referred to herein have been deposited

with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

5        Samples of the deposited plasmids will become available to the public upon grant of a patent based upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Samples of the deposited plasmids will be  
10 replaced if the depository is unable to dispense viable samples. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or  
15 similar plasmids that encode similar or equivalent antigens as described in this application are within the scope of the invention.

	<u>Plasmid</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
	pXL5	209143	July 16, 1997
20	pXL6	209144	July 16, 1997

#### EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific  
25 Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although  
30 specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, and immunology used but not explicitly  
35 described in this disclosure and these Examples are



amply reported in the scientific literature and are well within the ability of those skilled in the art.

#### Example 1

This Example describes the construction of vectors  
5 containing the RSV G gene.

Figure 1 shows a restriction map of the gene encoding the G protein of respiratory syncytial virus and Figure 2 shows the nucleotide sequence of the gene encoding the full-length RSV G protein (SEQ ID No: 1)  
10 and the deduced amino acid sequence (SEQ ID No: 2). Figure 3 shows the gene encoding the secreted RSV G protein (SEQ ID No: 3) and the deduced amino acid sequence (SEQ ID No: 4).

Plasmid pXLS (Figure 4) was prepared for the  
15 expression of the full-length RSV G protein as follows:

A recombinant Bluescript plasmid (RSV G12) containing the cDNA encoding the full-length G protein of a clinical RSV isolate (subgroup A) was used to construct vectors for RSV DNA-G immunization. RSV G12  
20 was digested with AflIII and EcoRI and filled-in with the Klenow subunit of DNA polymerase. The resulting 1.23 kb fragment containing the coding sequence for the full-length G protein was gel-purified and ligated to VR-1012 (Vical) (Figure 6 previously linearized with  
25 EcoRV. This procedure placed the RSV G cDNA downstream of the immediate-early cytomegalovirus (CMV) promoter and Intron A sequences of human cytomegalovirus (CMV) and upstream of the bovine growth hormone (BGH) poly-A site. The junctions of the cDNA fragments in the plasmid  
30 construct were confirmed by sequencing analysis. The resulting plasmid was designated pXLS.

Plasmid pXLS (Figure 5) was prepared for the expression of a secretory RSV G protein as follows:

RSV G12 was digested with EcoRI, filled-in with  
35 Klenow and digested again with BamHI. The BamHI

cleavage resulted in the generation of a cDNA fragment encoding a RSV G protein with N-terminal truncation. This DNA segment was gel-purified and ligated in the presence of a pair of 11 mer oligodeoxynucleotides  
5 (5'GATCCACTCAG 3') (SEQ ID no: 7)

3' GTGAGTCCTAG 5' (SEQ ID no: 8)

to VR-1020 (Vical) previously digested with BglII, filled in with Klenow, digested again with BamHI and gel-purified. This procedure placed the truncated RSV G  
10 cDNA (lacking the coding region for the N-terminal 91 amino acid residues including the transmembrane domain) downstream of the immediate-early CMV promoter and Intron A sequences of human CMV and upstream of the BGH poly-A site. In addition, there was the introduction of  
15 approximately 100 bp of 5' untranslated region and the coding sequence for the signal peptide of human plasminogen activator protein (Figure 7) fused in frame to the N-terminus of the RSV G protein coding sequence downstream of the CMV promoter/Intron A sequences. The  
20 junctions of the cDNA fragments in the plasmid construct were confirmed by sequencing analysis. The resulting plasmid was designated pXL6.

#### Example 2

This Example describes the immunization of mice.  
25 Mice are susceptible to infection by RSV as described in ref. 24.

Plasmid DNA was purified through double CsCl centrifugations. For intramuscular (i.m.) immunization, tibialis anterior muscles of BALB/c mice (male, 6 to 8  
30 week old) (Jackson Lab., Bar Harbor, ME, USA) were bilaterally injected with 2 x 50µg (1µg/µL in PBS) of either pXL5, pXL6 or V-1012. Five days prior to DNA injection, the muscles were treated with 2 x 50µL (10µM in PBS) of cardiotoxin (Latoxan, France) to increase DNA  
35 uptake and enhance immune responses, as reported by

Davis et al (ref. 23). The animals were boosted with the same dose of plasmid DNA 6 weeks and 13 weeks later, respectively. For intradermal (i.d.) immunization, 100µg of the plasmid DNA (2µg/µL in PBS) of were  
5 injected at the base of the tail and boosted 6 weeks and 13 weeks later, respectively. Mice in the positive control group were immunized intranasally (i.n.) with  $10^6$  plaque forming units (pfu) of a clinical RSV strain of the A2 subtype grown in Hep2 cells kindly provided by  
10 Dr. B. Graham (ref. 24).

Four weeks after the third immunization, mice were challenged intranasally with  $10^6$  pfu of the RSV A2 strain. Lungs were aseptically removed 4 days later, weighed and homogenized in 2 mL of complete culture  
15 medium (ref. 25). The number of pfu in lung homogenates was determined in duplicate as previously described (ref. 26) using vaccine-quality Vero cells.

#### Example 3

This Example describes the immunogenicity and  
20 protection by polynucleotide immunization.

Antisera obtained from immunized mice were analyzed for anti-RSV G IgG antibody titres using specific enzyme-linked immunosorbent assay (ELISA) and for RSV-specific plaque-reduction titres. ELISAs were performed  
25 using 96-well plates coated with immunoaffinity-purified RSV G protein (50 ng/mL) and 2-fold serial dilutions of immune sera. A goat anti-mouse IgG antibody conjugated to alkaline phosphatase (Jackson ImmunoRes., Mississauga, Ontario, Canada) was used as secondary  
30 antibody. Plaque reduction titres were determined according to Prince et al (ref. 26) using vaccine-quality Vero cells. Four-fold serial dilutions of immune sera were incubated with 50 pfu of the RSV Long strain (ATCC) in culture medium at 37°C for 1 hr in the  
35 presence of 5% CO<sub>2</sub> and the mixtures were used to infect

Vero cells. Plaques were fixed with 80% methanol and developed 5 days later using a mouse anti-RSV F monoclonal IgG1 antibody and donkey anti-mouse IgG antibody conjugated to peroxidase (Jackson ImmunoRes., 5 Mississauga, Ontario, Canada). The RSV-specific plaque reduction titre was defined as the dilution of serum sample yielding 60% reduction in plaque number. Both ELISA and plaque reduction assays were performed in duplicate and data are expressed as the means of two 10 determinations.

The results obtained are reproduced in Tables I and II below:

Table I. Immunogenicity of DNA-G in BALB/c Mice

Immunogen Titre	Anti-RSV G IgG Titre (Log 2(titre/100))			RSV-Specific Plaque Reduction (Log 2 titre) 17 weeks
	6 weeks	10 weeks	17 weeks	
VR-1012 (i.m.)	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.00 \pm 0.00$
pXL5 (i.m.)	$3.10 \pm 2.77$	$9.70 \pm 1.06$	$8.60 \pm 1.17$	$5.40 \pm 1.65$
pXL6 (i.m.)	$5.78 \pm 1.20$	$9.30 \pm 0.82$	$8.89 \pm 1.54$	$7.26 \pm 0.82$
pXL5 (i.d.)	$1.50 \pm 1.27$	$8.60 \pm 1.43$	$8.30 \pm 1.25$	$7.92 \pm 0.59$
pXL6 (i.d.)	$3.70 \pm 1.25$	$10.30 \pm 1.06$	$9.44 \pm 1.24$	$6.92 \pm 0.94$
RSV (i.n.)	$6.83 \pm 0.41$	$9.67 \pm 0.52$	$9.83 \pm 0.41$	$11.80 \pm 0.08$

*Table II. Immunoprotective Ability of DNA-G in BALB/c Mice*

<i>Immunogen</i>	<i>No. Mice</i>	<i>Mean Virus Lung Titre* (pfu/g lung) (Log 10 <math>\pm</math> SD)</i>	<i>No. Fully Protected Mice#</i>
VR-1012 (i.m.)	6	4.81 $\pm$ 0.01	0
pXL5 (i.m.)	6	0.29 $\pm$ 0.90	5
pXL6 (i.m.)	6	0.40 $\pm$ 1.20	5
pXL5 (i.d.)	6	0.30 $\pm$ 1.10	5
pXL6 (i.d.)	6	0.29 $\pm$ 0.90	5
RSV (i.n.)	6	0.00 $\pm$ 0.00	6

\*Sensitivity of the assay:  $10^{1.96}$  pfu/g lung

# The term, fully protected mice, refers to animals with no detectable RSV in the lungs 4 days post viral challenge.

As seen in Table I, plasmids pXL5 and pXL6 were found to be immunogenic following either i.m. or i.d. immunization producing anti-G antibodies and virus neutralizing antibodies. In addition, as seen in Table II, the plasmids pXL5 and pXL6 protected immunized mice against primary RSV infection of the lower respiratory tract. The control vector produced no immune response and did not confer protection.

#### Example 4

10 This Example describes the determination of the local lung cytokine expression profile in mice immunized with pXL5 and pXL6 after RSV challenge.

BALB/c mice were immunized at 0 and 6 weeks with 100µg of pXL5 and 6, prepared as described in Example 1, and challenged with RSV i.n. at 10 weeks. Control animals were immunized with placebo PI-RSV and live RSV and challenged with RSV according to the same protocol. In addition, animals were immunized with pXL2, as described in copending United States Patent Application no. 08/476,397 filed June 7, 1995 (WO 96/40945) and challenged with RSV, also following the same protocol. Four days post viral challenge, lungs were removed from immunized mice and immediately frozen in liquid nitrogen. Total RNA was prepared from lungs homogenized in TRIzol/β-mercaptoethanol by chloroform extraction and isopropanol precipitation. Reverse transcriptase-polymerase chain reaction (RT-PCR) was then carried out on the RNA samples using either IL-4, IL-5 or IFN-γ specific primers from Clontech. The amplified products were then liquid-hybridized to cytokine-specific <sup>32</sup>P-labeled probes from Clontech, resolved on 5% polyacrylamide gels and quantitated by scanning of the radioactive signals in the gels. Three mouse lungs were removed from each treatment group and analyzed for lung cytokine expression for a minimum of two times. The

data is presented in Figure 8 and represents the means and standard deviations of these determinations.

As may be seen from the data presented in Figure 8:

- 5        1. Immunization with live RSV intranasally (i.n.) resulted in a balanced cytokine profile (IFN- $\gamma$ , IL-4 and IL-5), whereas that with FI-RSV intramuscularly (i.m.) resulted in a Th2 predominance (elevated IL-4 and IL-5). These results are similar to those reported in the literature.
- 10       2. Immunization with pXL5 or pXL6 via either the i.m. or intradermal (i.d.) route gave rise to a balanced cytokine profile similar to that with live RSV immunization.
- 15       3. The magnitude of the cytokine responses with i.m. pXL6 (RSV G) and pXL2 (RSV F) immunization using the construct expressing a secretory form of the protein (SEC) is significantly higher than that with live RSV immunization.
- 20       4. The magnitude of the cytokine response with pXL5 immunization using constructs expressing a full-length membrane-associated RSV G protein (MA) and i.d. pXL6 was somewhat higher than that with live RSV immunization.
- 25       5. The balanced local cytokine response observed with DNA-G immunization contrasts with that reported by Openshaw et al (ref. 13). Using a recombinant vaccinia virus expressing the G protein, these investigators reported a local Th2 response by analysis of bronchoalveolar lavage. The results herein, which were obtained through a monogenic approach, indicate that the Th2 response is not necessarily an intrinsic property of the G protein.
- 30
- 35



SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides certain novel non-replicating vectors  
5 containing genes encoding RSV G proteins, methods of immunization using such vectors and methods of diagnosis using such vectors. Modifications are possible within the scope of this invention.

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CLAIMS

What we claim is:

1. An immunogenic composition for in vivo administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a non-replicating vector comprising:
  - a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
  - a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and
  - a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host, and
  - a pharmaceutically-acceptable carrier therefor.
2. The composition of claim 1 wherein said first nucleotide sequence encodes a full-length RSV G protein.
3. The composition of claim 2 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).
4. The composition of claim 2 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein having the amino acid sequence shown in Figure 2 (SEQ ID NO:2).
5. The composition of claim 1 wherein said first nucleotide sequence encodes a RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.
6. The composition of claim 5 wherein said non-replicating vector further comprises a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of said first nucleotide

sequence.

7. The composition of claim 6 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

8. The composition of claim 5 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).

9. The composition of claim 5 wherein said first nucleotide sequence comprises a nucleotide sequence encoding a truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID NO:4).

10. The composition of claim 1 wherein said promoter sequence is a immediate early cytomegalovirus promoter.

11. The composition of claim 1 wherein said second nucleotide sequence is the human cytomegalovirus Intron A.

12. The composition of claim 1 wherein the non-replicating vector is a plasmid vector.

13. The composition of claim 12 wherein the plasmid vector is pXL5 as shown in Figure 4.

14. The composition of claim 13 wherein the plasmid vector is pXL6 as shown in Figure 5.

15. A method of immunizing a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to said host an effective amount of a non-replicating vector comprising:

a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from

said vector in the host.

16. The method of claim 15 wherein said first nucleotide sequence encodes a full-length RSV G protein.

17. The method of claim 16 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).

18. The method of claim 16 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein shown in Figure 2 (SEQ ID NO:2).

19. The method of claim 14 wherein said first nucleotide sequence encodes an RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.

20. The method of claim 19 wherein said non-replicating vector further comprises a heterologous signal peptide encoding nucleotide sequences immediately upstream of the 5'-terminus of said first nucleotide sequence.

21. The method of claim 20 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

22. The method of claim 19 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).

23. The method of claim 19 wherein said first nucleotide sequence comprises a nucleotide sequence encoding a transverse RSV G protein shown in Figure 3 (SEQ ID NO:4).

24. The method of claim 15 wherein said promoter sequence is an immediate early cytomegalovirus promoter.

25. The method of claim 15 wherein said second nucleotide sequence is the human cytomegalovirus Intron A.

26. The method of claim 1 wherein the non-replicating vector is a plasmid vector.

27. The method of claim 26 wherein said plasmid vector is pXL5 as shown in Figure 4.

28. The method of claim 26 wherein said vector is pXL6 as shown in Figure 5.

29. The method of claim 15 wherein a balanced Th1/Th2 immune response is induced.

30. A method of using a gene encoding a respiratory syncytial virus (RSV) G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, to produce an immune response in a host, which comprises:

isolating said gene,

operatively linking said gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of said RSV G protein when introduced into a host to produce an immune response to said RSV G protein, and

introducing said vector into a host.

31. The method of claim 30 wherein said gene encoding an RSV G protein encodes a full length RSV G protein.

32. The method of claim 30 wherein said gene encoding an RSV G protein encodes an RSV G protein lacking the transmembrane domain and sequences upstream thereto.

33. The method of claim 32 wherein said vector further comprises a signal peptide encoding nucleotide sequences immediately upstream of the 5'-terminus of said first nucleotide sequence.

34. The method of claim 33 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

35. The method of claim 30 wherein said at least one control sequence comprises the immediate early cytomegalovirus promoter.

36. The method of claim 35 including the step of:

operatively linking said gene to an

immunoprotection enhancing sequence to produce an enhanced immunoprotection to said RSV G protein in said host.

37. The method of claim 36 wherein said immunoprotection enhancing sequence is introduced into said vector between said control sequence and said gene.

38. The method of claim 37 wherein said immunoprotection enhancing sequence is the human cytomegalovirus Intron A.

39. The method of claim 30 wherein said gene is contained within a plasmid selected from the group consisting of pXL5 and pXL6.

40. A method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isolating a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

operatively linking said first nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of said RSV G protein when introduced to a host to produce an immune response to said RSV G protein,

operatively linking said first nucleotide sequence to a second nucleotide sequence to increase expression of said RSV G protein in vivo from the vector in the host, and

formulating said vector as a vaccine for in vivo administration to a host.

41. The method of claim 40 wherein said vector is selected from group consisting of pXL5 and pXL6.

42. A vaccine produced by the method of claim 40.

43. A method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample,



comprising the steps of:

- (a) immunizing a host with a non-replicating vector to produce antibodies specific for the RSV G protein, said non-replicating vector comprising:
  - a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
  - a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and
  - a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host,
- (b) isolating the RSV G protein specific antibodies;
- (c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in a sample and said isolated RSV G protein-specific antibodies; and
- (d) determining the production of the complexes.

44. The method of claim 43 wherein said vector is selected from the group consisting of pXL5 and pXL6.

45. A diagnostic kit for detecting the presence of a respiratory syncytial virus RSV G protein in a sample, comprising:

- (a) a non-replicating vector capable of generating antibodies specific for the RSV G protein when administered to a host, the non-replicating vector comprising:

- a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

- a promoter sequence operatively coupled to

said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host;

(b) isolation means to isolate said RSV G protein-protein-specific antibodies;

(c) contacting means to contact the isolated RSV G specific antibodies with the sample to produce a complex comprising any RSV G protein in the sample and RSV G protein specific antibodies, and

(d) identifying to determine production of the complex.

46. The diagnostic kit of claim 45 wherein said vector is selected from the group consisting of pXL5 and pXL6.

47. A method for producing antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising:

(a) immunizing a host with an effective amount of a non-replicating vector to produce RSV G-specific antibodies, said non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host; and

(b) isolating the RSV G-specific antibodies from the host.

48. A method of producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising the steps of:

(a) constructing a vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host;

(b) administering the vector to at least one mouse to produce at least one immunized mouse;

(c) removing B-lymphocytes from the at least one immunized mouse;

(d) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(e) cloning the hybridomas;

(f) selecting clones which produce anti-RSV G protein antibody;

(g) culturing the anti-RSV G protein antibody-producing clones; and then

(h) isolating anti-RSV G protein antibodies from the cultures.

1 / 19

## Restriction Map of the RSV G Gene

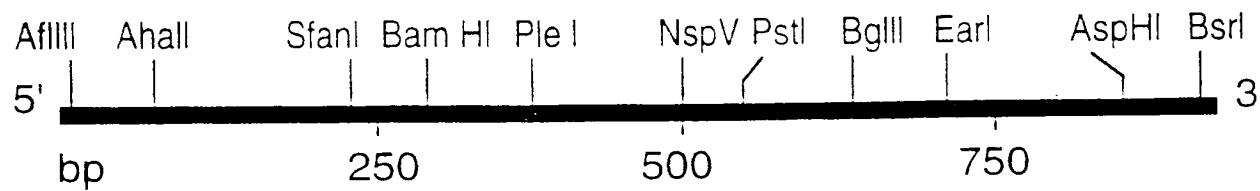


FIG.1

2/19

## FIG.2A

10	19	28	37	46	55
TGCAAC ATG TCC AAA AAC AAG GAC CAA GGC ACC GGT AAG ACA CTA GAA AAG ACC					
Met Ser Lys Asn Lys Asp Gln Arg Thr Ala Lys Thr Leu Glu Lys Thr					
64	73	82	91	100	109
TGG GAC ACT CTC AAT CAT TTA TTA TTC ATA TCA TCG GGC TTA TAT AAG TTA AAT					
Trp Asp Thr Leu Asn His Leu Leu Phe Ile Ser Ser Gly Leu Tyr Lys Leu Asn					
118	127	136	145	154	163
CTT AAA TCT GTA GCA CAA ATC ACA TTA TCC ATT CTG GCA ATG ATA ATC TCA ACT					
Leu Lys Ser Val Ala Gln Ile Thr Leu Ser Ile Leu Ala Met Ile Ile Ser Thr					
172	181	190	199	208	217
TCA CTT ATA ATT ACA GGC ATC ATA TTC ATA GGC TCG GAA AAC CAC AAA GTC ACA					
Ser Leu Ile Ile Thr Ala Ile Ile Phe Ile Ala Ser Ala Asn His Lys Val Thr					
226	235	244	253	262	271
CTA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG ATC AAG AAC ACA ACC CCA					
Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser Ser Gln Ile Lys Asn Thr Thr Pro					
280	289	298	307	316	325
ACA TAC CTC ACT CAG GAT CTT CAG CTT GGA ATC AGC TTC TCC AAT CTG TCT GAA					
Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser Asn Leu Ser Glu					

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## FIG.2B

334	343	352	361	370	379
ATT ACA TCA CAA ACC ACC ACC ATA CTA GCT TCA ACA ACA CCA GTC AAG TCA					
Ile Thr Ser Gln Thr Thr Thr Ile Leu Ala Ser Thr Thr Pro Gly Val Lys Ser					
388	397	406	415	424	433
AAC CTG CAA CCC ACA ACA GTC AAG ACT AAA AAC ACA ACA ACC CAA ACA CAA					
Asn Leu Gln Pro Thr Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Gln Thr Gln					
442	451	460	469	478	487
CCC AGC AAG CCC ACT ACA ACA CAA CCG CAA AAC AAA CCA AAC AAA CCC AAT					
Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn					
496	505	514	523	532	541
AAT GAT TTT CAC TTC GAA GIG TTT AAC TTT GTA CCC TGC AGC ATA TGC AGC AAC					
Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn					
550	559	568	577	586	595
AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA AAA CCA GGA AAG					
Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly Lys					
604	613	622	631	640	649
AAA ACC ACC AAG CCT ACA AAA CCA ACC TTC AAG ACA ACC AAA AAA GAT					
Lys Thr Thr Thr Lys Pro Thr Thr Lys Lys Pro Thr Phe Lys Thr Thr Lys Lys Asp					

## FIG.2C

658	667	676	685	694	703
CTC AAA CCT CAA ACC AGT AAA CCA AAG GAA GTA CCC ACC ACC AAC CCC ACA CAA					
Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Lys Pro Thr Glu					
712	721	730	739	748	757
GAG CUA ACC ATC AAC ACC ACC AAA AUA AAC ATC ACA ACT ACA CTG CTC ACC AAC					
Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn Ile Thr Thr Thr Leu Leu Thr Asn					
766	775	784	793	802	811
AAC ACC ACA GCA AAT CCA AAA CTC ACA AGT CAA ATG GAA ACC TTC CAC TCA ACC					
Asn Thr Thr Gly Asn Pro Lys Lys Thr Ser Gln Met Glu Thr Phe His Ser Thr					
820	829	838	847	856	865
TCC TCC GAA GGC AAT CTA AGT CCT TCT CAA GTC TTC ACA ACA TCC GAG CAC CCA					
Ser Ser Gln Gly Gly Asn Leu Ser Pro Ser Gln Val Ser Thr Thr Ser Glu His Pro					
874	883	892	901	914	
TCA CAA CCC TCA TCT CCA CCC AAC ACA ACA CGC CAG TAGTATTATA AAAAAAAAAA					
Ser Gln Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln .					

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## FIG.3A

CAC AAA GTC ACA CTA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG ATC AAG	54
His Lys Val Thr Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser Gln Ile Lys	18
AAC ACA ACC CCA ACA TAC CTC ACT CAG GAT CCT CAG CTT GGA ATC AGC TTC TTC	108
Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser	36
AAT CTG TCT GAA ATT ACA TCA CAA ACC ACC ACC ATA CTA GCT TCA ACA ACA CCA	162
Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr Thr Thr Thr Thr Thr Thr Thr	54
GGA GTC AAG TCA AAC CTG CAA CCC ACA ACA GTC AAG ACT AAA AAC ACA ACA ACA	216
Gly Val Lys Ser Asn Leu Gln Pro Thr Thr Thr Val Lys Thr Lys Asn Thr Thr Thr	72
ACC CAA ACA CAA CCC AGC AAG CCC ACT ACA AAA CAA CCC CAA AAC AAA CCA CCA	270
Thr Gln Thr Gln Pro Ser Lys Pro Thr Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro	90
AAC AAA CCC AAT AAT GAT TTT CAC TTC GAA GIG TTT AAC TTT GTA CCC TGC AGC	324
Asn Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser	108
ATA TGC AGC AAC AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA	378
Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys	126
AAA CCA GGA AAG AAA ACC ACC AAG CCT ACA AAA AAA CCA ACC TTC AAG ACA	432
Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys Thr	144

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FIG.3B

ACC AAA AAA GAT CTC AAA CTT CAA ACC ACT AAA CCA AAG GAA GGA CUC ACC ACC 486  
 Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr 162

AAG CUC ACA GAA GAG CCA ACC AUC AAC ACC AAA ACA AAC ATC ACA ACT ACA 540  
 Lys Pro Thr Glu Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn Ile Thr Thr 180

CIG CTC ACC AAC AAC ACC ACA GAA AAT CCA AAA CUC ACA AGT CAA AUG GAA ACC 594  
 Leu Leu Thr Asn Asn Thr Thr Gly Asn Pro Lys Leu Thr Ser Gln Met Glu Thr 198

TTC CAC TCA ACC TUC TUC GAA GUC AAT CTA ACC CTT TCT CAA GUC TUC ACA ACA 648  
 Phe His Ser Thr Ser Ser Glu Gly Asn Leu Ser Pro Ser Gln Val Ser Thr Thr 216

TUC GAG CAC CCA TCA CAA CUC TCA TCT CCA CUC AAC ACA CUC CAG TAG 699  
 Ser Glu His Pro Ser Gln Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln 232

TTTATTAA AAAAAAAAAA

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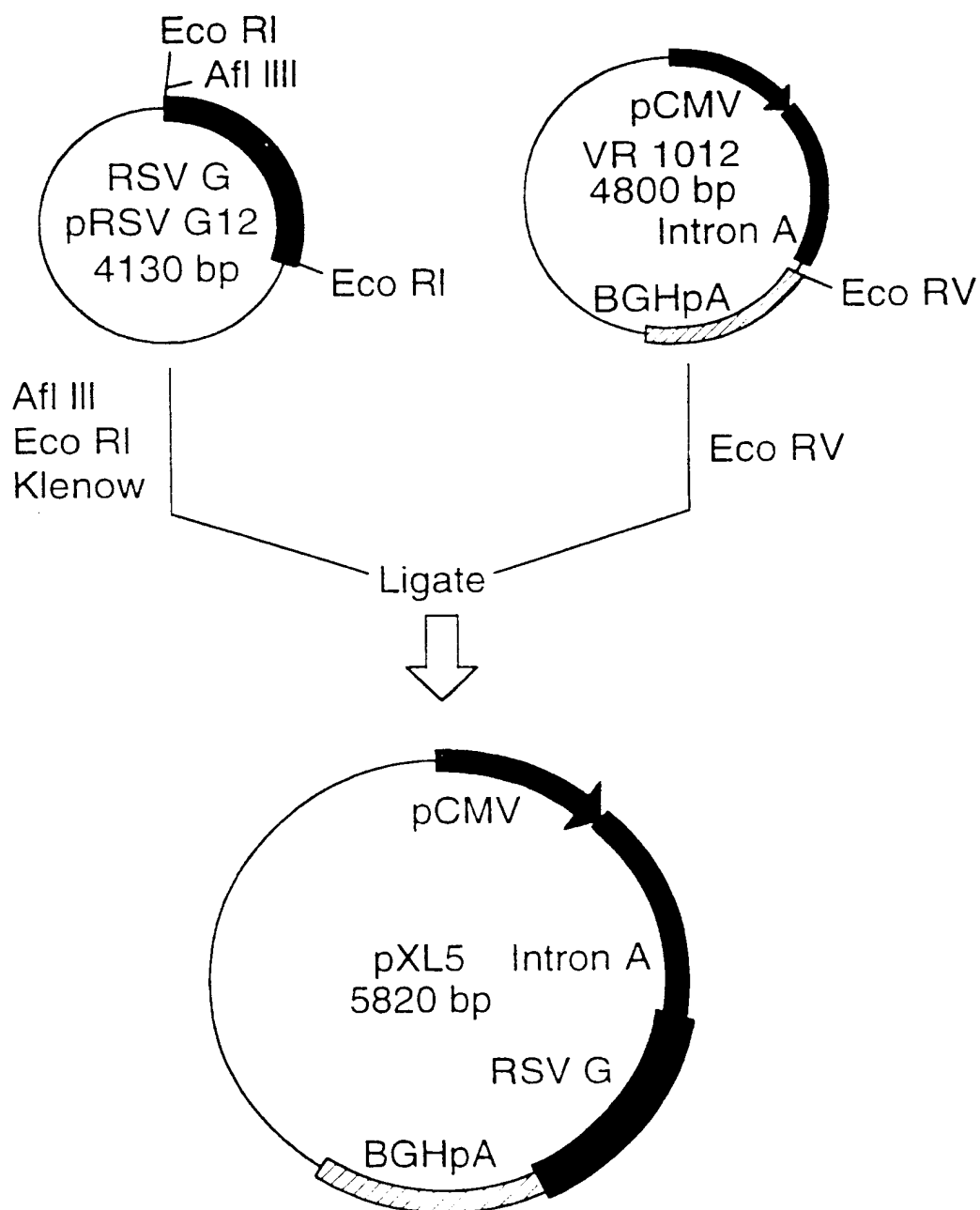


FIG.4.

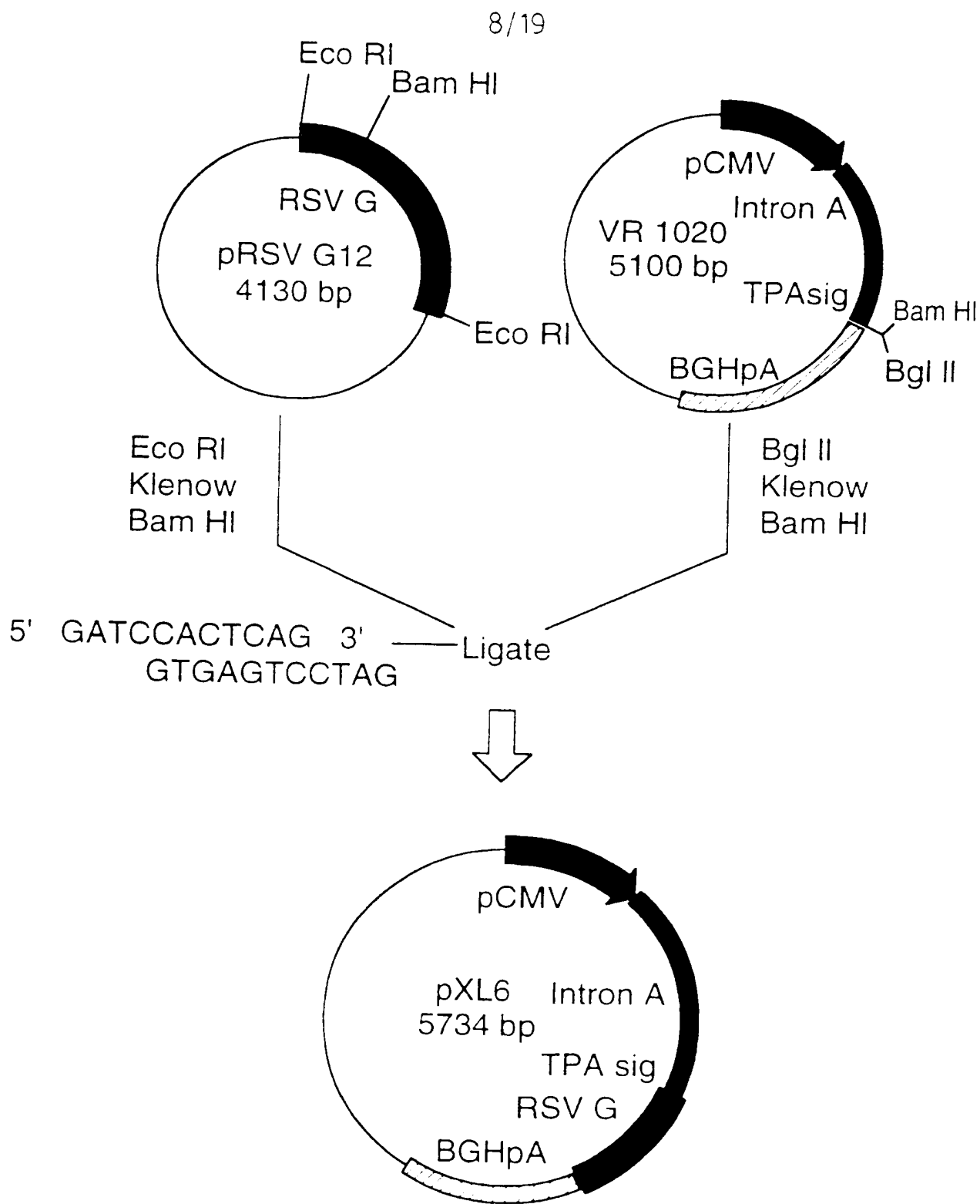


FIG.5

FIG. 6A

10 20 30 40 50 60 70  
 TCGCGCGTTT CCGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA CAGCTTGTCT

80 90 100 110 120 130 140  
 GTAAGCGGAT GCGCGGAGCA GACAAGCCCG TCAGCGCGGTC TTGCGCGGTG TCGCGGCTCG

150 160 170 180 190 200 210  
 CTTAACTATG CCGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATCCG GTCGAAATA CCGCACAGAT

220 230 240 250 260 270 280  
 GCGTAAGGAG AAAATACCC ATCAGATTGG CTATTGCCA TTGCATACGT TGTATCCATA TCATAATATG

290 300 310 320 330 340 350  
 TACATTATTA TTGGCTCATG TCCAACATTA CCGCCATGTT GACATTGATT ATTGACIAGT TATTAAATAGT

360 370 380 390 400 410 420  
 AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCGCGGTT ACATAACTTA CCGTAAATGG

430 440 450 460 470 480 490  
 CCGGCGCTGC TGACCGCCCA ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAAACG

500 510 520 530 540 550 560  
 CCAATAGCGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACCGTAAAC TGCCCACTTG GCAGTACATC

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FIG.6B

570 580 590 600 610 620 630  
 AAGTGTATCA TATGCCAAGT ACCTGCTTATA TTGACGTCAA TGACGCTAAA TGCCCGCGCT GGCATTATGC

640 650 660 670 680 690 700  
 CCAGTACATG ACCTTATGCG ACCTTCTTAC TTGCTAGTAC ATTCTAGTAT TAGTCTATGCT TATTACCTATG

710 720 730 740 750 760 770  
 GTCATGCGCT TTGCTGAGTA CATTCAATGCG CGTCTATAC GCTTTCTA TC ACCGCTATTT CCAAGCTCTC

780 790 800 810 820 830 840  
 ACCCATATTA CGTCAATGCG AGTTTCTTTT GCTACCAAAA TCAACCTTAC TTTCCTAAAAT GTCTTAACTA

850 860 870 880 890 900 910  
 CTGCTGCTTA TTCTGCTTAA TGCTCTGCTG GCTCTGCTG TGCTCTGCTT ATATATACCTAG AGCTCTGCTTAA

920 930 940 950 960 970 980  
 GTCTACCTGTC AGATGCTGTC GATCACTCTAT CCACGCTGTT TTCTACCTCTTA TAGTACGACAC CCGGACCGAT

990 1000 1010 1020 1030 1040 1050  
 CCACGCTGTC CGCTCTCTAA CGCTCTCTCT GACCTCTCTAT TCCCTCTCTCT AAGAGCTCTAG TAAGTACCTCT

1060 1070 1080 1090 1100 1110 1120  
 CTATAGACTC TATAGGCACA CCCCCTTCTGCT TCCTATGCTAT GCTATACCTG TTTTCTCTCT GGGCTCTATAC

FIG.6C

1130 1140 1150 1160 1170 1180 1190  
 ACCCGCGCTT CCTTATGCTA TAGATCATGG TATAGCTTAG CCTATAGGIG TGGGTTATIG ACCATTATIG  
  
 1200 1210 1220 1230 1240 1250 1260  
 ACCACTCCCC TATTGGIGAC GATACTTTCC ATTACTAATC CATACATATGG CTCCTTTGGCA CAACTATCTC  
  
 1270 1280 1290 1300 1310 1320 1330  
 TATTGGCTAT ATGCCAATAC TCCTGCTCTC AGAGACTGAC ACCTACCTCTG TATTTTITACA GGATGGGGTC  
  
 1340 1350 1360 1370 1380 1390 1400  
 CCATTATTA TTTACAAATT CACATATACA ACAACCTCTT CTTCTCTCTT CCAGTTTTT ATTAAACATA  
  
 1410 1420 1430 1440 1450 1460 1470  
 GCGTGGGATC TOCAGCCGAA TCTTCTCTAC GTGTCTCTGA CATCTCTCTT TCTCCGGTAG CCGCGGAGCT  
  
 1480 1490 1500 1510 1520 1530 1540  
 TCCACATCG AGCCCTGGTC CCATGCCCTCC AGCGGCTCAT GGTCTCTCTG CAGCTCTCTG CTCTAACAG  
  
 1550 1560 1570 1580 1590 1600 1610  
 TGGAGGCCAG ACTTAGGCAC AGACAATGC CCACCACCAC CAGTCTCTCG CACAAGGCCG TGGCGGTAGG  
  
 1620 1630 1640 1650 1660 1670 1680  
 GTATGCTCT GAAATGAGC GTGGAGATIG GCTTCGCAG GCTGACCGAG ATGCAAGACT TAAGCCAGCG

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FIG.6D

1690	1700	1710	1720	1730	1740	1750
GCAGAAAG	ATGCAGGCAG	CTGAGTGT	GTATTCTGAT	AAGAGTCAGA	GTPAACTCC	GTTGGGGTTC
1760	1770	1780	1790	1800	1810	1820
TGTTAACTGT	GTAGGGAGT	GTAGTCTGAG	CAGTACTGT	TGTTGTTGG	CGGCCACCA	GACATAATAG
1830	1840	1850	1860	1870	1880	1890
CTTACAGACT	AACAGACTGT	TCTTTTCCAT	GGGTCTTTTC	TGCAGTCACC	GTCGTGCACA	CGTGTGATCA
1900	1910	1920	1930	1940	1950	1960
GATATCGCG	CCGCTCTAGA	CCAGGGCTT	GGATCCAGAT	CTGCTGTGCC	TTCCTAGTTC	CAGCCATCTG
1970	1980	1990	2000	2010	2020	2030
TTCCTTGACC	CTCCCCCGTG	CCCTTCTCTGA	CCCTGGAAG	TGCACATCC	ACTGTCTCTT	CCTAATAAAA
2040	2050	2060	2070	2080	2090	2100
TGAGGAATT	GCATCGCAAT	GTCTGAGTAG	GTCATCTCT	ATTCTGGGG	GTCGGTGG	GCAGGACAC
2110	2120	2130	2140	2150	2160	2170
AACGGGAGG	ATTGGGAAGA	CAATAGCAG	CATCTCTGGG	ATCGGTGG	CTCTATGGT	ACCCAGGTTC
2180	2190	2200	2210	2220	2230	2240
TGAAGAATTG	ACCCGGTTC	TCTTGGGCA	GAAAGAAGCA	GGCACAATCC	CTTCTCTGTG	ACACACCCCTG

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FIG.6E

2250            2260            2270            2280            2290            2300            2310  
 TTCAGGCCC TGGTCTTAG TTCCAGCCC ACTCATAGCA CACTCATAGC TCAGGAGGGC TCGGCTTCA  
  
 2320            2330            2340            2350            2360            2370            2380  
 ATCCACCCG CTAAAGTACT TGGAGCGGTC TTCTCCCTCCC TCATCAGCCC ACCAAACCAA ACCTAGCCTC  
  
 2390            2400            2410            2420            2430            2440            2450  
 CAAGAGTGG AAGAAATTAA ACCAAGATAG GCTATTAAAGT GCAGAGGGAG AGAAATGCC TCCAACATGT  
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 2460            2470            2480            2490            2500            2510            2520  
 GAGGAAGTAA TGAGAGAAAT CATAGAAATT CTTCGGCTTC CTGGCTACT GACTGGCTGC GCTGGTGTG  
  
 2530            2540            2550            2560            2570            2580            2590  
 TCGGCTGCG CGAGCGGTAT CAGCTCACTC AAAGCGGTA ATACGGTTAT CCACAGAATC AGCGGATAAC  
  
 2600            2610            2620            2630            2640            2650            2660  
 GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACGGTAA AAAGGCGCG TTGCTGGCGT  
  
 2670            2680            2690            2700            2710            2720            2730  
 TTTTTCATAG GCTCGGCCCC CCTGACGAGC ATCACAAAAA TCGACCTCA AGTCAGAGGT GCGGAAACCC  
  
 2740            2750            2760            2770            2780            2790            2800  
 GACAGGACTA TAAAGATACC AGCGTTTCC CCGTGAAGC TCCCTGCTGC GCCTCTGTG TCGACCCCTG



FIG.6F

2810	2820	2830	2840	2850	2860	2870
CCGCTTACCG	GATACCTGTC	CCCCCTTCTC	CCTTCGGGA	GGGUGGCGCT	TTCTCATAGC	TCACGCTGTA
2880	2890	2900	2910	2920	2930	2940
GGTATCTCAG	TTCCGGGTAG	GTCGTTCCGT	CCAAGCTCCG	CCTGTCTGCAC	GAACCCCGCG	TTTACAGCTGA
2950	2960	2970	2980	2990	3000	3010
CCGCTGCGCC	TTATCCCGTA	ACTATCTGCT	TGAGTCCAAC	CCCGTAAGAC	ACGACTTATC	GCCACTGQCA
3020	3030	3040	3050	3060	3070	3080
GCAGCCACTG	GTAACAGCAT	TAGCAGAGCG	AGGTATGTAG	GGGTTCTTAC	AGAGTTCTTG	AAGTGGTQCC
3090	3100	3110	3120	3130	3140	3150
CTAACTACCG	CTACACTACA	AGAACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA
3160	3170	3180	3190	3200	3210	3220
AAGAGTTGGT	AGCTCTTTGAT	CCGGCAACA	AACCACCGCT	GGTAGCCGIG	GT'TTTTTTGT	TTGCAAGCAG
3230	3240	3250	3260	3270	3280	3290
CAGATTACGC	GCAGAAAAA	AGGATCTCAA	GAAGATCCIT	TGATCTTTTC	TACGGCGTCT	GACGCTCAGT
3300	3310	3320	3330	3340	3350	3360
GGAACGAAA	CTGACGTTAA	GGCATTTTGG	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	AGATCTTTT

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## FIG.6G

3370	3380	3390	3400	3410	3420	3430
AAATTAAAA	TGAAGTTT	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC
3440	3450	3460	3470	3480	3490	3500
TTAATCAGIG	AGGCACCTAT	CTCAGCGATC	TGTCATTTTC	GTTCATCCAT	AGTTCCTTGA	CTCGGGGGGG
3510	3520	3530	3540	3550	3560	3570
GGGGGGGGCIG	AGGTCIGCCT	CGTGAAGAAG	GIGTTCCTGA	CTCATATACCAG	GGCTGAAATCG	CCCCATCATC
3580	3590	3600	3610	3620	3630	3640
CAGCCAGAAA	GTCAGGGAGC	CACGGTTGAT	GAGAGCTTTG	TTGTAGGTCG	ACCAGTTGGT	GATTTTGAAC
3650	3660	3670	3680	3690	3700	3710
TTTTCCTTTG	CCACGGAAACG	GCTTCGGTTG	TCGGGAAGAT	GGGTGATCTG	ATCCTTCAAC	TCAGCAAAAG
3720	3730	3740	3750	3760	3770	3780
TTTCGATTTAT	TCAACAAAAC	CGCCGTCCTG	TCAAGTCAGC	GTPATGCTCT	GGCAGTGTGA	CAACCAATTA
3790	3800	3810	3820	3830	3840	3850
ACCAATTGTG	ATTAGAAAAA	CTCATTCGAGC	ATCAAATGAA	ACTGCAATTT	ATTTCATATCA	GGATTATCAA
3860	3870	3880	3890	3900	3910	3920
TACCATATTT	TTGAAAAAAGC	CGTTTCTCTGA	ATGAAGGAGA	AAACTCACC	AGGCAGTTC	ATAGGATGCC

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## FIG.6H

3930	3940	3950	3960	3970	3980	3990
AATATCCTGG	TATCGGCTG	CGATTCCGAC	TCGTCCAACA	TCAATACAAC	CTATTATATT	CCCCCTGTC
4000	4010	4020	4030	4040	4050	4060
AAAATAAGGT	TATCAAGTCA	CAAATCACCA	TGAGTGACGA	CTGAATCCCG	TGAGAATGGC	AAAAGCTTAT
4070	4080	4090	4100	4110	4120	4130
GCATTTCCTT	CCAGACTTGT	TCAACAGGCC	AGCCATTACG	CTCGTCATCA	AAATCACTCG	CATCAACCAA
4140	4150	4160	4170	4180	4190	4200
ACCGTTATTC	ATTCTGICATT	GCTGCTTGAGC	GACGACGAAT	ACCGGATCGC	TGTTAAAGG	ACAATTACAA
4210	4220	4230	4240	4250	4260	4270
ACAGGAATCG	AATGCAACCG	GCGCAGCAAC	ACTGCCAGCG	CATCAACAAT	ATTTTCACCT	GAATCAGGAT
4280	4290	4300	4310	4320	4330	4340
ATTCTTCTAA	TACCTGGAAT	GCTGTCTTTC	CGGGGATCGC	AGTGGTGAGT	AACCATGCGAT	CATCAGGAGT
4350	4360	4370	4380	4390	4400	4410
ACCGATAAAA	TGCTTGATGG	TCGGAAGAGG	CATAAATTCC	GTCAGCCAGT	TTAGTCTGAC	CATCTCATCT
4420	4430	4440	4450	4460	4470	4480
GTAACATCAT	TGGCAACGGT	ACCCTTGCCA	TGTTTCAGAA	ACAACCTCTGG	CGCATCGGGC	TTCOCATACA

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## FIG. 6I

4490 4500 4510 4520 4530 4540 4550  
 ATCGATAGAT TGTCGCACCT GATTGCCCCG CATTATCGCG AGCCCATTTA TACCCATATA AATCAGCATC  
  
 4560 4570 4580 4590 4600 4610 4620  
 CATGTTGGAA TTTAATCGCG GCGTCGAGCA AGACGTTTCC CGTTGAATAT GGCCTCATAAC GTTCCCTTGTA  
  
 4630 4640 4650 4660 4670 4680 4690  
 TTACTGTTTA TGTAAGCAGA CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC  
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 4700 4710 4720 4730 4740 4750 4760  
 AGAGATTTTG AGACACAACG TGGCTTTTCC CCCCCCCCCA TTATTGAAGC ATTTATCAGG GTTATTGTCT  
  
 4770 4780 4790 4800 4810 4820 4830  
 CATGAGCGGA TACATATTTG AATGTAATTA GAAAAATAAA CAAATAGGGG TTCCGGCCAC ATTTCGCCGA  
  
 4840 4850 4860 4870 4880 4890 4900  
 AAAGTCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TAAAAATAGG CGTATACACGA  
  
 4910  
 GGGCCCTTTCG TC

FIG.7

10	20	30	40	50	60	70
CTGCAGTCAC	CGTCGTGGAC	CAGAGCTGAG	ATCCTACAGG	AGTCCAGGGC	TCGAGAGAAA	ACCTCTGGGA
80	90	100	110	120	130	140
GGAAAGGGAA	GGAGCAAGCC	GTCGAATTTAA	GGGACGCTGT	GAAGCAATCA	TCGATGCCAAT	GAAAGAGAGGG
150	160	170	180			
CTCTGCTGIG	TGCTGCTGCT	GTGTGGAGCA	GTCCTTGTTT	CGCCGAGC		

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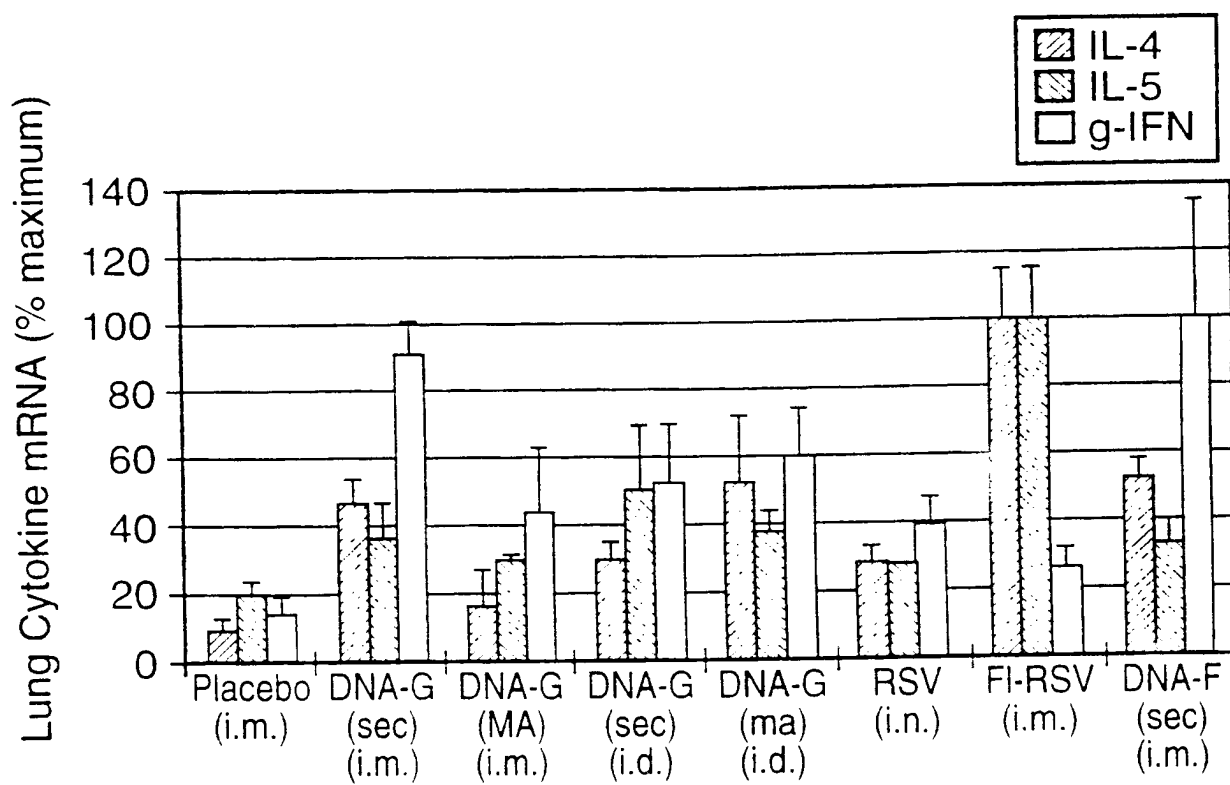


FIG.8

# INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/CA 98/00697

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/45 A61K48/00 G01N33/53 C07K16/10

According to International Patent Classification, IPC, into both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched: classification system followed by classification symbols

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search: name of data base and, where practical, search terms used:

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category: Citation of document with indication, where appropriate, of the relevant passages Relevant to claim No

X	STOTT E J ET AL: "Human respiratory syncytial virus glycoprotein G expressed from a recombinant vaccinia virus vector protects mice against live-virus challenge." JOURNAL OF VIROLOGY, (1986 NOV) 60 (2) 607-13. JOURNAL CODE: KCV. ISSN: 0022-538X. XP002080963 United States see page 607 see abstract see page 608 'Results', first paragraph see page 609: figure 1 ---	30-35
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☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex

### Special categories of cited documents

- A document defining the general state of the art which is not considered to be of particular relevance
- E earlier document but published on or after the international filing date
- L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- O document referring to an oral disclosure, use, exhibition or other means
- P document published on or after the international filing date but later than the priority date claimed

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- S document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

15 October 1998

30/10/1998

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Sitch, W

## INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/CA 98/00697

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>HSU K H ET AL: "Immunogenicity of recombinant adenovirus- respiratory syncytial virus vaccines with adenovirus types 4, 5, and 7 vectors in dogs and a chimpanzee." JOURNAL OF INFECTIOUS DISEASES. (1992 OCT) 166 (4) 769-75. JOURNAL CODE: IH3. ISSN: 0022-1899.. XP002080964 United States see page 769 see abstract</p>	30-35
X	<p>STOTT E J ET AL: "Immune and histopathological responses in animals vaccinated with recombinant vaccinia viruses that express individual genes of human respiratory syncytial virus." JOURNAL OF VIROLOGY. (1987 DEC) 61 (12) 3855-61. JOURNAL CODE: KCV. ISSN: 0022-538X.. XP002080965 United States see page 3855 see abstract see page 3856. 'Materials and Method', first and second paragraphs</p>	30-35
X	<p>ELANGO N ET AL: "Resistance to human respiratory syncytial virus ( RSV ) infection induced by immunization of cotton rats with a recombinant vaccinia virus expressing the RSV G glycoprotein." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. (1986 MAR) 83 (6) 1906-10. JOURNAL CODE: PV3. ISSN: 0027-8424.. XP002080966 United States see page 1906 see abstract see page 1907; figure 1</p>	30-35
A	<p>ROBERTS S R ET AL: "The membrane-associated and secreted forms of the respiratory syncytial virus attachment glycoprotein G are synthesized from alternative initiation codons." JOURNAL OF VIROLOGY. (1994 JUL) 68 (7) 4538-46. JOURNAL CODE: KCV. ISSN: 0022-538X.. XP002080967 United States see page 4538 see abstract see page 4541; figure 1</p>	5,6,8,9, 19,20, 22,23, 28,32,33

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# INTERNATIONAL SEARCH REPORT

Intern      II Application No.  
PCT/CA 98/00697

## C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim no.
A	<p>NGUYEN T N ET AL: "Hydrophobicity engineering to facilitate surface display of heterologous gene products on <i>Staphylococcus xylosus</i>." JOURNAL OF BIOTECHNOLOGY, (1995 OCT 16) 42 (3) 207-19. JOURNAL CODE: AL6. ISSN: 0168-1656.. XP000561319 Netherlands see page 207 see abstract</p> <p>---</p>	<p>5,6,8,9, 19,20, 22,23, 28,32,33</p>
A	<p>WATHEN M W ET AL: "Characterization of a novel human respiratory syncytial virus chimeric FG glycoprotein expressed using a baculovirus vector." JOURNAL OF GENERAL VIROLOGY, (1989 OCT) 70 ( PT 10) 2625-35 JOURNAL CODE: 19B. ISSN: 0022-1317 . XP002080968 ENGLAND: United Kingdom see page 2625 see abstract</p> <p>---</p>	<p>5,6,8,9, 19,20, 22,23, 28,32,33</p>
A	<p>US 5 620 896 A (HERRMANN JOHN E ET AL) 15 April 1997 see column 2, line 62 - column 5, line 31; figures 1A,1B,3,4A,4B</p> <p>---</p>	<p>1-29, 36-48</p>
P.X	<p>SCHRIJVER R S ET AL: "Comparison of DNA application methods to reduce BRSV shedding in cattle" VACCINE, vol. 16, no. 2-3, 2 January 1998, page 130-134 XP004098613 see the whole document</p> <p>---</p>	<p>1-48</p>
P.X	<p>JOHNSON T R ET AL: "Priming with secreted glycoprotein G of respiratory syncytial virus ( RSV ) augments interleukin-5 production and tissue eosinophilia after RSV challenge." JOURNAL OF VIROLOGY, (1998 APR) 72 (4) 2871-80. JOURNAL CODE: KCV. ISSN: 0022-538X.. XP002080969 United States see page 2871 see abstract</p> <p>-----</p>	<p>30-35</p>

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/ 00697

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 15-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat. Application No.

PCT/CA 92/00697

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